Cytologic Detection of Esophageal Squamous Cell Carcinoma and Precursor Lesions Using Balloon and Sponge Samplers in Asymptomatic Adults in Linxian, China

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BACKGROUND. The principal reason for the poor prognosis of esophageal carcinoma is that most tumors are asymptomatic and go undetected until they are unresectable. Previous studies have shown that cytologic screening of asymptomatic high risk individuals can detect curable esophageal carcinomas and precursor lesions, but the sensitivity of such screening is not well documented. The current study evaluated the sensitivity and specificity of currently available balloon and sponge cytologic samplers for detecting biopsy-proven squamous dysplasia and carcinoma in asymptomatic individuals from a high risk population in Linxian, China.

METHODS. Asymptomatic adults were examined with both balloon and sponge samplers, in random order, followed by endoscopy with mucosal iodine staining and biopsy of all unstained lesions. The cytology slides were interpreted using the criteria of the Bethesda System. The balloon and sponge cytologic diagnoses (test) were compared with the biopsy diagnosis (truth) in each patient to estimate the sensitivity and specificity of each sampler.

RESULTS. Of the 439 patients with adequate biopsies, 123 (28%) had histologic squamous dysplasia and 16 (4%) had an invasive squamous carcinoma. The sensitivities/specificities of the balloon and sponge were 44%/99% and 18%/100%, respectively, for detecting biopsy-proven squamous cell carcinoma, and 47%/81% and 24%/92%, respectively, for identifying squamous dysplasia or carcinoma.

CONCLUSIONS. In this study, the balloon sampler was more sensitive than the sponge sampler for detecting esophageal squamous disease, but both techniques were less than optimal. Improved samplers and/or cytologic criteria should increase the sensitivities observed in this baseline study. ***Cancer*** **1997;80:2047–59.**
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Esophageal carcinoma is estimated to cause 300,000 deaths in the world each year.[1] In China, ''esophageal carcinoma,'' including

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tumors of the esophagus and gastric cardia, is the second leading cause of cancer death.[2] In the U.S., esophageal carcinoma causes approximately 10,000 deaths each year, and is the fourth most common cause of cancer death in African-American men and the eighth leading cause of cancer death in men of all races.[3] In 1983–1990, the overall 5-year survival rate for esophageal carcinoma in the U.S. was 9.2%, among the lowest of all cancers.[3]

The most important single factor in the prognosis of esophageal carcinoma is the extent of disease at the time of diagnosis.[4,5] Patients with Stage I tumors (T1N0M0), invading only the lamina propria or submucosa without lymph node or distant metastases,[6] have a 90% 5-year survival after resection,[4,5] but $<1\%$ of patients in most series are diagnosed with Stage I disease.[7,8] The principal reason for poor survival in patients with esophageal carcinoma is that most tumors are asymptomatic and go undetected until they have spread beyond the esophageal wall. Significant reduction of esophageal carcinoma mortality will probably require new strategies for earlier detection and treatment of curable precursor and early invasive esophageal lesions.

Early detection of curable esophageal lesions will require the screening of asymptomatic high risk individuals. A successful early detection and treatment program will need an accurate, cost-effective, and patient-acceptable primary screening test; a secondary test that can confirm and localize all neoplastic lesions; and curative therapy that is acceptable to asymptomatic individuals. Such a screening program should be applicable in high risk populations both in China and in other countries.

The most common primary screening technique for the early detection of esophageal carcinoma is cytologic screening of high risk populations. Two principal types of cytologic samplers have been used in these screenings: an inflatable balloon sampler developed in China[9–12] and an encapsulated sponge sampler developed in Japan.[13–16] Previous studies of both of these samplers have reported high sensitivities (73–99%) for detecting esophageal carcinoma in symptomatic patients.[9–16] However, there is little published information regarding the accuracy of either of these methods in asymptomatic individuals, who would comprise the target group for any population screening effort.

During the past decade, Chinese and American researchers have collaborated in the conduct of two nutrition intervention trials in Linxian, a high risk county in Henan Province in north-central China.[17] This research has allowed us to perform several preliminary studies relevant to the evaluation of balloon cytology as a screening technique for esophageal carcinoma in asymptomatic high risk individuals.[18] The results of these studies suggested that the current balloon technique may be only moderately sensitive for detecting dysplasia and carcinoma in these individuals. To obtain additional information, we performed a more detailed study to evaluate the ability of both the balloon and sponge samplers to detect biopsy-proven dysplasia and carcinoma in asymptomatic individuals from the high risk Linxian population.

MATERIALS AND METHODS

The general design of this cytologic-histologic correlation study was to perform both the balloon and sponge cytologic sampling techniques, in random order, on each patient, followed by endoscopy; to read all cytologic and histologic slides in a blinded fashion; and then to evaluate the ability of each cytologic sampler to detect biopsy-proven disease. This study was approved by the Institutional Review Boards of the collaborating institutions, the Cancer Institute of the Chinese Academy of Medical Sciences (CICAMS), and the U. S. National Cancer Institute.

Patient Population

Participants were recruited in the spring of 1995 from 11 villages in Linqi Commune, an administrative district in the south of Linxian. Although Linxian is well known for its high rates of esophageal carcinoma, and northern Linxian has been the site of many previous studies, the inhabitants of Linqi Commune previously had not been studied in a systematic manner by cytologic or endoscopic techniques. All residents age 50–69 years in the 11 villages were asked to participate, unless they had a history of cirrhosis, esophageal varices, vomiting blood, or a reaction to topical anesthetics or iodine, or were considered too weak to undergo the examinations.

Cytologic Examinations

All cytologic examinations were performed in the patients' villages, at a health clinic or a community center. The patients were instructed to fast overnight before the examinations. After completing a short questionnaire to document symptoms and the absence of contraindicated conditions, the patients were directed to a balloon or sponge examination station according to a computer-generated random order list. As soon as the patients completed their first examination, they were sent to the other examination station for their second exam. Each of the two exams was assigned a different, randomly generated cytology case number. Incomplete examinations, the patient's grading of balloon and sponge procedural discomfort, and the pa-

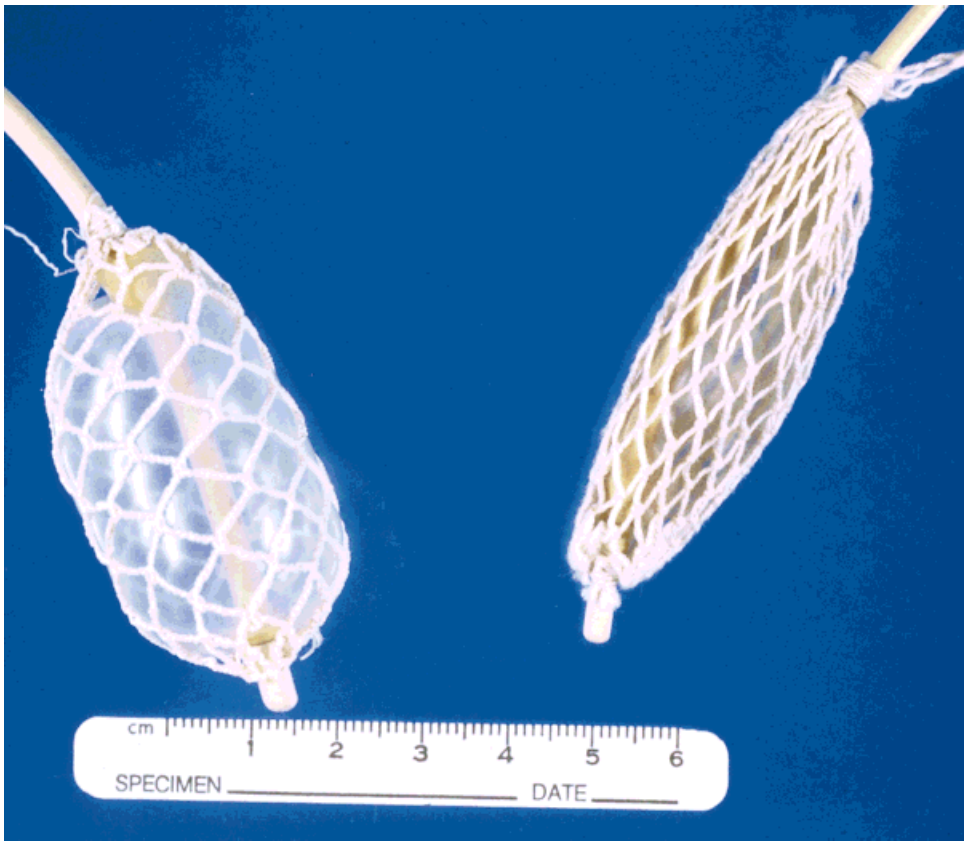

FIGURE 1. The inflatable balloon sampler used in this study (Fifth Rubber Manufacturing Co., Shanghai, China).

tient's preference of samplers were recorded on a patient acceptability form.

Balloon method

The balloon sampler used in this study (Fig. 1) was a rubber balloon covered with a cotton mesh net and attached to a double lumen rubber tube (Fifth Rubber Manufacturing Co., Shanghai, China). The inner lumen of the tube passed through the center of the balloon to allow suction of cells from an obstructing lesion and the outer lumen was used for balloon inflation. The collapsed balloon was 1.2 cm in diameter and 5.0 cm long. Inflated with 20–30 mL of air, it was 2.5–3.0 cm in diameter and 5.0 cm long. This balloon sampler, originally designed by Dr. Shen Qiong of Henan Medical University, has been used in most of the mass population screenings performed in China during the last 30 years.

Before examination, dental prostheses were removed and the mouth was rinsed with water. The patient was given 2 mL of a 2% lidocaine slurry by mouth for local anesthesia. With the patient sitting upright, a technician inserted the balloon into the back of the throat and the patient was asked to swallow. In many cases, the technician could assist the passage of the balloon past the upper sphincter and through the esophagus into the stomach. Once in the stomach, the balloon was inflated with 20–30 mL of air, and then gradually pulled back up the esophagus. The amount of air was adjusted to match the resistance to the bal-

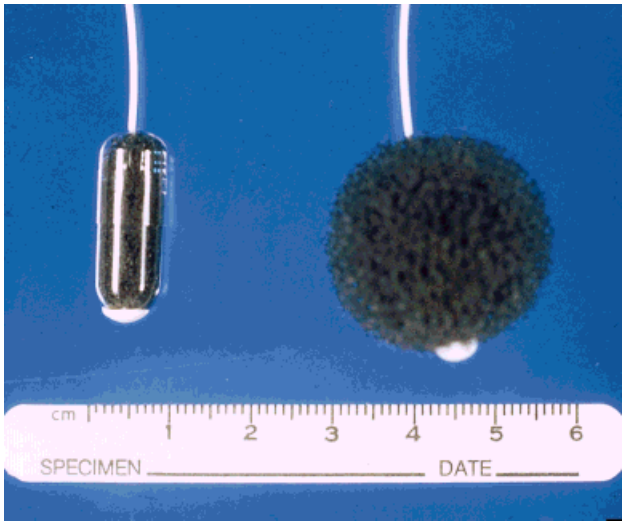

FIGURE 2. The encapsulated sponge sampler used in this study (Cellmate®, Biosearch Medical Products, Inc., Somerville, NJ).

loon's passage. When the balloon reached 18 cm from the incisor teeth, all air was released and the balloon was withdrawn completely. After removal, the balloon was smeared directly onto four slides, which were labeled only with the patient's balloon cytology case number and a consecutive number (1–4) indicating the order in which the slides were smeared. The slides were immediately fixed in 95% ethanol for 15 minutes, and later stained with Papanicolaou stain.

Sponge method

The sponge sampler used in this study (Fig. 2) was a sphere of polyurethane mesh compressed inside a gelatin capsule and attached to a thin solid plastic stylet (Cellmate®; Biosearch Medical Products, Inc., Somerville, NJ). The compressed, encapsulated sponge measured 0.8 cm in diameter and was 2.3 cm long. Expanded, it was a 2.5-cm sphere. This sampler was similar in design to the encapsulated sponge first introduced by Dr. Kinichi Nabeya and used for esophageal screening in Japan.[13,14] Dr. Nabeya's sponge, which had a coarser texture and was attached to a string, was not available at the time of this study.

Before examination, dental prostheses were removed and the mouth was rinsed with water. With the patient sitting upright, a technician placed the encapsulated sponge in the back of the throat and the patient was asked to swallow the capsule with water. The plastic stylet was too pliable for the technician to assist in passing the capsule past the upper sphincter, but once in the esophagus the capsule could be threaded into the stomach by advancing the stylet, with sips of water as needed. The sponge was left in the stomach for 5 minutes, to allow the gelatin capsule to dissolve and the sponge to expand, after which it was pulled up the esophagus. The sponge was shaken in 30 mL of Saccomanno's solution (50% ethanol, and 2% Carbowax™) and then discarded. The cell suspension then was centrifuged at 1500 revolutions per minute for 5 minutes. The supernatant fluid was discarded by inversion, and the pellet resuspended in the remaining supernatant fluid. This concentrated cell suspension was smeared onto four slides, labeled only with the patient's sponge cytology case number and a consecutive number (1–4) indicating the order in which the slides were smeared. The slides were air dried and later stained with Papanicolaou stain.

Endoscopic Examinations

The patients underwent endoscopy in May 1995, approximately 1 month after their cytology examinations. Because all the study participants were farmers, the time available for the endoscopic examinations was limited by the beginning of the local winter wheat harvest. Because there was insufficient time to endoscopically examine all subjects, priority was given to those who had completed both cytology examinations. Endoscopy was performed at a CICAMS field station in Yaocun Commune, approximately 50 km north of Linqi. The examinations were done before the cytology slides were read, so the endoscopist was unaware of the cellular adequacy or diagnosis of the cytology smears.

After an overnight fast, the patients were brought by van to the field station, and consecutive endoscopy case numbers were assigned. The patients were given 5 mL of a 1% dicaine slurry by mouth for local anesthesia 2–5 minutes before endoscopy, but were not otherwise sedated. Endoscopy was performed using an Olympus GIF-130 videoendoscope (Olympus Corporation, Tokyo, Japan). After insertion of the endoscope, the entire esophagus and stomach were examined, and all visible lesions were described and photographed. Then 20–30 mL of 1.2% glycerine free Lugol's iodine solution (12 gm iodine + 24 gm potassium iodide in 1000 mL water) was sprayed from the gastroesophageal junction to the upper esophageal sphincter using a spray catheter (washing tube PW-5L; Olympus Corporation) passed through the biopsy channel.[19] Lugol's iodine stains normal squamous mucosa brown, but leaves foci of squamous dysplasia and carcinoma and all glandular mucosa unstained.[20] After iodine spraying, unstained areas in the esophagus were described and photographed. One or more 2.8-mm biopsies were taken from each unstained area and from at least 1 normally stained midesophageal site. Gastric biopsies were taken only when visible lesions were observed before staining.

Cytology Slide Reading

The cytology smears of the patients who underwent endoscopy were read by one of the authors (M.J.R., S.F.L., C.C., B.Z., or D.S.). The cytology slides were labeled only with the cytology case number and a consecutive number (1–4) representing the order in which each slide in the case had been smeared. There was no way for the readers to link the two cytology case numbers or the cytology and endoscopy case numbers of an individual patient.

Because our previous studies in Linxian had suggested that Western cytologic categories might be somewhat more predictive than Chinese categories,[18] we chose to use diagnostic categories and criteria adapted from criteria of the Bethesda system.[21,22] An evaluation of specimen adequacy, a squamous diagnosis, and a glandular diagnosis were recorded for each of the four slides in each case. The overall speci-

men adequacy was a cumulative evaluation of all slides in the case. The overall squamous and glandular diagnoses were the worst squamous and glandular diagnoses observed in the case. Cytology lesions diagnosed as atypical squamous cells of undetermined significance (ASCUS), atypical glandular cells of undetermined significance (AGUS), or higher were reviewed for quality assurance by a second reviewer familiar with the Bethesda system (M.J.R. or D.S.). If a discrepancy existed between the first and the second reviewers, the second reviewer's interpretation superseded that of the first.

A few modifications of the Bethesda system categories and criteria were made prior to slide reading based on the esophageal cytology experience of the readers. The nuclear enlargement allowed in specimens diagnosed as Benign Reactive Cellular Changes was increased from 2 to 2.5 times the area of a normal intermediate squamous cell nucleus. A Suspicious for Carcinoma category was added to include cases with high grade cellular changes, occasional visible nucleoli, and a suggestion of a tumor diathesis. A Not Otherwise Specified (NOS) designation was used for cases with highly atypical, usually immature cells that could not be classified definitely as either squamous or glandular. These cases were classified as High Grade Intraepithelial Lesion NOS, Suspicious for Carcinoma NOS, or Carcinoma NOS.

Biopsy Slide Reading

The endoscopic biopsy slides were read by one of the authors (M.J.R. or S.M.D.), using criteria previously described,[23,24] without knowledge of the cytology results.

Analysis

For each patient, the overall squamous and glandular cytologic diagnoses of each sampler (test) were compared with the worst squamous and glandular endoscopic biopsy diagnoses (truth) to give separate estimates of the ability of each sampler to detect biopsy-proven squamous and glandular disease. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for each cytologic sampler for each site. Squamous and glandular diagnoses were analyzed separately, and, because the balloon and sponge were designed to sample only the esophagus and gastric cardia, only biopsies from these sites were included in the evaluation. Because >90% of the cells on the cytology slides were squamous cells, the NOS diagnoses were combined with the squamous diagnoses for analysis.

The analyses were performed at three diagnostic levels: carcinoma/no carcinoma (CA/no CA); high grade dysplasia or worse/no high grade dysplasia (HGD+/no HGD); and dysplasia or worse/no dysplasia (DYS+/no DYS). The HGD+ level was chosen for separate analysis because a previous study in Linxian showed that individuals with biopsy-proven high grade (moderate or severe) squamous dysplasia were the only ones at significantly increased risk for developing invasive squamous carcinoma within 3.5 years after biopsy.[25] For these evaluations, the following groupings of cytologic and histologic diagnoses were used:

Diagnostic level	Cytologic categories included	Histologic categories included
Carcinoma	Carcinoma Suspicious for Carcinoma	Carcinoma
HGD+	Carcinoma Suspicious for Carcinoma High Grade Squamous Intraepithelial Lesion (HSIL)	Carcinoma Severe Squamous Dysplasia Moderate Squamous Dysplasia High Grade Glandular Dysplasia
DYS+	Carcinoma Suspicious for Carcinoma HSIL Low Grade Squamous Intraepithelial Lesion (LSIL) ASCUS, favor neoplastic AGUS, favor neoplastic	Carcinoma Severe Squamous Dysplasia Moderate Squamous Dysplasia Mild Squamous Dysplasia High Grade Glandular Dysplasia Low Grade Glandular Dysplasia

In addition to these primary analyses, other evaluations were performed to investigate the influence of sampler order, the occurrence of learning curves among sampler technicians or slide readers, the necessity of reading four slides per case, and the effect of varying the cytologic criteria included in the diagnostic levels.

To investigate the influence of sampler order, the squamous cytology-histology comparisons were repeated for the balloon when it was the first sampler, the balloon when it was the second sampler, the sponge when it was the first sampler, and the sponge when it was the second sampler.

To examine the possibility of improvements over time (learning curves) among the sampler technicians or the slide readers, the cases were divided into halves and quarters based on the cytology sampling date or the date that the slides were read and the squamous cytology-histology comparisons were repeated on cases included in each subgroup.

To evaluate the usefulness of examining four slides per case, the cytology-histology comparisons were repeated as they would have been if only one, two, or three slides had been smeared in each case. This was done by creating separate data files with the

TABLE 1
Patient Acceptance of the Screening Examinations

Sampler	No. of subjects	Complete examinations	Patient preference[a]
Balloon	625	620 (99%)	165 (31%)
Sponge	538	462 (86%)	231 (43%)
No preference	NA	NA	142 (26%)

[a] Among patients who attempted both examinations (N = 538).

TABLE 2
Adequacy of the Cytologic Samples

Sampler	No. of cases[a]	No. with adequate squamous cells	No. with adequate glandular cells
Balloon	434	431 (99%)	338 (78%)
Sponge	378	326 (86%)	29 (8%)

[a] Number of cases in the analytic cohort (N = 439) who completed the balloon or sponge examinations.

TABLE 3
Cytology Results

Squamous diagnoses[a]		
Diagnosis	**Balloon[b] no. (%)[c]**	**Sponge[d] no. (%)**
Unsatisfactory for evaluation of squamous cells	3 (1)	52 (14)
Within Normal Limits	56 (13)	143 (38)
Benign Reactive Cellular Changes	158 (36)	93 (25)
ASCUS, favor Reactive	97 (22)	40 (11)
ASCUS, favor Neoplastic	62 (14)	24 (6)
LGSIL	42 (10)	23 (6)
HGSIL	5 (1)	0 (0)
Suspicious for Carcinoma	4 (1)	3 (1)
Squamous Cell Carcinoma	7 (2)	0 (0)
Total	434	378
Glandular diagnoses[e]		
Diagnosis	**Balloon[f] no. (%)**	**Sponge[g] no. (%)**
Unsatisfactory for evaluation of glandular cells	85 (20)	333 (92)
Within Normal Limits	192 (45)	24 (7)
Benign Reactive Cellular Changes	94 (22)	0 (0)
ASCUS, favor Reactive	37 (9)	3 (1)
ASCUS, favor Neoplastic	12 (3)	2 (1)
Suspicious for Carcinoma	1 (0.2)	0 (0)
Adenocarcinoma	2 (0.5)	0 (0)
Total	423	362

ASCUS: atypical squamous cells of undetermined significance; LGSIL: low grade squamous intraepithelial lesion; HGSIL: high grade squamous intraepithelial lesion.

[a] Worst squamous cytology diagnoses in subjects in the analytic cohort (N = 439). These diagnoses included one balloon case and one sponge case originally termed High-Grade Intraepithelial Lesion not otherwise specified (NOS), one balloon case and one sponge case originally termed Suspicious for Carcinoma NOS, and 3 balloon cases originally termed Carcinoma NOS.

[b] Five subjects missing: unsuccessful balloon examination in five cases.

[c] Number of subjects (column percent).

[d] Sixty-one subjects missing: sponge examination not attempted in 10 cases; unsuccessful sponge examination in 51 cases.

[e] Worst glandular cytology diagnoses in subjects in the analytic cohort (N = 439).

[f] Sixteen subjects missing: unsuccessful balloon examination in 5 cases; no glandular cytology result in 11 cases.

[g] Seventy-seven subjects missing: sponge examination not attempted in 10 cases; unsuccessful sponge examination in 51 cases; no glandular cytology result in 16 cases.

information from Slide 1, from Slides 1 and 2, and from Slides 1, 2, and 3 of each cytology case and repeating the cytology-histology comparisons on these files.

To examine the effect of varying the cytologic criteria, we repeated the cytology-histology comparisons with different cytologic categories included in the diagnostic levels of HGD+ and DYS+.

Differences in independent proportions were tested using the normal approximation to the binomial distribution.[26] Differences in correlated proportions were tested using a McNemar test.[27]

The analytic cohort for this cytology-histology correlation study was comprised of the 439 individuals who underwent endoscopy and had at least 1 satisfactory endoscopic biopsy. In all individual cytology-histology comparisons, the number of subjects analyzed was comprised of all patients who completed the cytologic examination and had a satisfactory endoscopic biopsy from the tissue type being evaluated.

RESULTS

Six hundred and twenty-five individuals agreed to participate in this study, including 549 individuals in the targeted 50–69-year age group. There were 307 men and 318 women, with an average age of 60 years and an age range of 26–78 years. On questioning, 23 individuals (3.7%) admitted difficulty swallowing, 9 (1.4%) reported pain on swallowing, 26 (4.2%) had at least 1 of these symptoms, and 6 (1.0%) had both.

All 625 participants attempted a balloon examination. However, during the last days of the screening the supply of single-use sponges ran out, so only 538 patients attempted a sponge examination. Because of time constraints related to the beginning of the local winter wheat harvest, only 459 patients underwent endoscopy, including 373 who had completed both cytology examinations.

Table 1 shows the completion rates of the two cytologic examinations and the sampler preferences of those who attempted both techniques. A significantly greater proportion of patients completed the balloon

TABLE 4
Endoscopic Biopsy Results

Worst squamous diagnosis[a]	
Diagnosis	**No. (%)[b]**
Normal	254 (58)
Esophagitis	44 (10)
Mild dysplasia	52 (12)
Moderate dysplasia	45 (10)
Severe dysplasia	26 (6)
Squamous cell carcinoma	16 (4)
Total	437
Worst glandular diagnosis[c]	
Diagnosis	**No. (%)**
Normal	8 (20)
Gastritis, with or without atrophy	18 (45)
Low grade glandular dysplasia	2 (5)
High grade glandular dysplasia	4 (10)
Adenocarcinoma	8 (20)
Total	40

[a] Worst squamous biopsy diagnosis in subjects in the analytic cohort (N = 439). Two subjects had no satisfactory squamous biopsies.
[b] Number of subjects (column percent).
[c] Worst glandular biopsy diagnosis in subjects in the analytic cohort (N = 439). Three hundred ninety-nine subjects had no satisfactory glandular biopsies.

examination than completed the sponge examination (99% vs. 86%; $P < 0.01$). Reasons for incomplete sponge examinations included patient anxiety due to lack of familiarity with the sponge procedure and inability to swallow the sponge capsule. However, of the patients who attempted both examinations, a significantly greater percentage preferred the sponge sampler (43% vs. 31%; $P < 0.01$). Six percent of these patients graded the balloon examination and 4% graded the sponge examination as moderately or severely uncomfortable.

Table 2 shows the adequacy of the cell samples from the 439 patients in the analytic cohort. Nearly all of the balloon samples (99%) had many well visualized squamous cells, but only 86% of the sponge samples had enough squamous cells to be satisfactory for evaluation ($P < 0.01$). Seventy-eight percent of the balloon samples, but only 8% of the sponge samples, contained adequate numbers of glandular cells to be evaluated ($P < 0.01$).

The squamous and glandular cytology diagnoses for both samplers are shown in Table 3. In the squamous results, nearly 50% of the subjects were within normal limits when sampled by the sponge, but they had a much wider distribution of diagnoses when sampled by the balloon. The most striking finding in the glandular results was that insufficient numbers of glandular cells were obtained in >90% of the sponge samples.

All 459 patients who underwent endoscopy completed the procedure successfully. However, in 20 patients no satisfactory biopsies were obtained. There were no patients with Barrett's metaplasia, so all glandular biopsies were of gastric origin.

The endoscopic biopsy results are shown in Table 4. In the majority of cases (59%), all squamous biopsies were normal. Ten percent of cases had a worst squamous diagnosis of esophagitis, 12% had low grade (mild) dysplasia, 16% had high grade (moderate or severe) dysplasia, and 4% had invasive squamous cell carcinoma. Only 40 subjects who underwent endoscopy had glandular biopsies. Of these, 20% had only normal biopsies, 45% had gastritis, 5% had low grade dysplasia, 10% had high grade dysplasia, and 20% had invasive adenocarcinoma.

Table 5 shows the correlation of cytologic and endoscopic biopsy results, by cytology sampler, tissue type, and the three diagnostic levels of analysis. Evaluation of the balloon sampler's ability to detect gastric disease was limited by the small number of gastric biopsies, and evaluation of the sponge sampler's ability to identify gastric lesions was not possible because so few sponge samples contained glandular cells.

At all three diagnostic levels, the balloon sampler was more sensitive and less specific than the sponge for detecting biopsy-proven squamous disease. The sensitivities/specificities of the balloon and sponge were 44%/99% and 18%/100%, respectively, for detecting squamous cell carcinoma; 16%/99% and 4%/100%, respectively, for identifying high grade dysplasia or carcinoma; and 47%/81% and 24%/92%, respectively, for detecting any dysplasia or carcinoma. Only the differences in sensitivity for HGD+ ($P = 0.008$) and DYS+ ($P = 0.001$) and the difference in specificity for DYS+ ($P < 0.001$) were statistically significant.

The two samplers had similar predictive values for squamous disease at all three diagnostic levels. The positive predictive values of the balloon and sponge were 64% and 67% for CA/no CA, 88% and 100% for HGD+/no HGD, and 53% and 56% for DYS+/no DYS. The negative predictive values of the balloon and sponge were 98% and 98% for CA/no CA, 83% and 81% for HGD+/no HGD, and 77% and 73% for DYS+/no DYS.

Sampler order did not greatly influence the balloon results, but it made a significant difference in the sensitivity of the sponge. When the balloon was the first sampler, its sensitivities were 45%, 17%, and 49% for detecting squamous CA, HGD+, and DYS+, respectively; when it was the second sampler, its sensitivities

TABLE 5
Correlation of Cytologic Diagnoses (Test) and Endoscopic Biopsy Results (Truth)

		Squamous diagnoses		Glandular diagnoses
Analytic categories	**Statistic**	**Balloon N = 432[a]**	**Sponge N = 376[b]**	**Balloon N = 39[c]**
CA/No CA	Sensitivity	44% (16)[d]	18% (11)	25% (8)
	Specificity	99%	100%	100%
HGD+/No HGD	Sensitivity	16% (85)	4% (73)	17% (12)
	Specificity	99%	100%	100%
DYS+/No DYS	Sensitivity	47% (137)	24% (177)	29% (14)
	Specificity	81%	92%	100%

[a] Seven subjects missing: unsuccessful balloon examination in five cases; no satisfactory squamous biopsy in two other cases.
[b] Sixty-three subjects missing: sponge examination not attempted in 10 cases; unsuccessful sponge examination in 51 cases; no satisfactory squamous biopsy in 2 other cases.
[c] Four hundred subjects missing: unsuccessful ballon examination in 5 cases; no glandular cytology result in 11 cases; no satisfactory glandular biopsy in 384 other cases.
[d] Number of patients in the cohort with biopsy-proven squamous or glandular carcinoma, high grade dysplasia or worse, or dysplasia or worse.
CA: carcinoma; HGD+: high grade dysplasia or worse; DYS+: dysplasia or worse.

TABLE 6
Variation in Screening Characteristics Caused by Defining Different Cytologic Categories as a Positive Screening Test for Histologic Squamous HGD+

		Cytologic categories included in the diagnostic level of HGD+				
Sampler		**HSIL-CA**	**LSIL-CA**	**ASCUS/N-CA**	**ASCUS/R-CA**	**Reactive-CA**
Balloon	No.[a]	16 (4)	58 (13)	120 (28)	217 (50)	375 (87)
	Sensitivity	16%	40%	59%	80%	95%
	Specificity	99%	93%	80%	58%	16%
Sponge	No.	3 (1)	26 (7)	50 (13)	90 (24)	183 (49)
	Sensitivity	4%	18%	25%	38%	56%
	Specificity	100%	96%	89%	80%	53%

HGD+: high grade dysplasia or worse; HSIL: high grade squamous intraepithelial lesion; CA: carcinoma; LSIL: low grade squamous intraepithelial lesion; ASCUS/N: atypical squamous cells of undetermined significance, favor neoplasia; ASCUS/R: atypical squamous cells of undetermined significance, favor reactive.
[a] Number (%) of patients in the included cytologic categories.

for these lesions were 40%, 16%, and 44%, respectively ($P > 0.10$ for all comparisons). When the sponge was the first sampler, its sensitivities were 40%, 9%, and 34% for detecting squamous CA, HGD+, and DYS+, respectively; when it was the second sampler, its sensitivities for these lesions dropped to 0%, 0%, and 11%, respectively ($P = 0.09$, $P = 0.05$, and $P < 0.01$, respectively, for these comparisons). The sensitivities of the balloon and sponge were not significantly different when they were the first sampler ($P > 0.07$ for all comparisons).

There was no clear evidence of learning curves among sampler technicians or slide readers. The sensitivity of the balloon samples for detecting squamous DYS+ was 40% in the first half of the screening and 52% in the second half ($P = 0.16$). For the sponge, these figures were 26% and 22%, respectively. The sensitivity of the balloon samples for identifying squamous DYS+ was 44% when the smears were examined in the first half of the slide reading and 49% when they were evaluated in the second half. For the sponge samples, these figures were both 24%. Similar analyses of sensitivities for detecting squamous cell carcinoma and HGD+ and analyses of sensitivities for detecting squamous DYS+ by sampling or reading quarters were limited by small numbers of cases in each subgroup.

For both samplers, the majority of cases of squamous dysplasia and carcinoma were identified on the first slide reviewed, but additional cases were found on each additional slide that was examined. These findings were the same regardless of whether the slides were read in the order that they had been smeared. In the balloon samples, there were 38, 48, 58, and 64 cases of squamous DYS+ detected on the first, the first 2, the first 3, and all 4 slides, respectively, yielding sensitivities of 28%, 35%, 42%, and 47%, respectively.

All these sensitivity increments were statistically significant ($P \leq 0.01$). In the sponge samples, 18, 21, 25, and 28 cases of DYS+ were identified on 1, 2, 3, and 4 slides, respectively, yielding sensitivities of 15%, 18%, 21%, and 24%, respectively. None of these sensitivity increments were statistically significant ($P \geq 0.06$). Similar analyses of squamous cell carcinoma, squamous HGD+, and glandular lesions were limited by the small numbers of cases found on the cytology smears.

The effect of varying the cytologic categories included in the diagnostic level of squamous HGD+ is shown in Table 6. For both the balloon and the sponge, there was a large increase in sensitivity, with only a small reduction in specificity, for identifying biopsy-proven HGD+ when cases diagnosed cytologically as LSIL were included in the analytic category of HGD+. Including additional cytologic categories in the diagnostic level of DYS+ also increased the sensitivity of identifying histologic DYS+, but only at the expense of a large reduction in specificity (data not shown).

DISCUSSION

Esophageal carcinoma is a common malignancy with a very poor prognosis. The main reason for its poor prognosis is that most tumors are asymptomatic until they become unresectable. There is a clear need for practical techniques that can screen asymptomatic high risk individuals and identify curable precursor lesions and early invasive carcinoma.

The most common primary esophageal screening method that has been used to date is esophageal cytology, employing inflatable balloon or encapsulated sponge samplers. Recently, primary endoscopic screening has been reported from Japan[28] and France,[29] but widespread application of this method will probably be limited by its cost. Endoscopy with mucosal iodine staining is a very sensitive technique for identifying clinically significant squamous esophageal lesions,[30] but in most settings it will be more appropriate as a secondary test to confirm and localize lesions identified by a cheaper, less invasive primary screening method.

The accuracy of currently available esophageal cytologic screening methods is not well documented. Nearly all previous studies of the accuracy of the balloon and sponge samplers have been evaluations of the sensitivity of these techniques in symptomatic patients who were already known to have carcinoma. There are little published data regarding the accuracy of these methods in identifying unknown carcinomas or precursor lesions in an asymptomatic population. The current study was designed to estimate the sensitivity, specificity, and predictive values of a single balloon or sponge examination for identifying concurrent, biopsy-proven esophageal dysplasia or carcinoma in asymptomatic individuals from a high risk population. The purpose of this baseline study was to evaluate whether either of these currently available samplers has potential as a primary screening test in a clinically useful early detection program for esophageal carcinoma.

Highlights of the current study design included examination of most patients by both screening methods, which allowed for a direct comparison of sampler results; the use of Western cytologic criteria, which have not often been used in previous evaluations of these samplers; and the use of endoscopy with mucosal iodine staining, a very sensitive method for detecting the presence of squamous dysplasia or carcinoma, as the gold standard for the cytologic-histologic comparisons.

For several reasons, reliable data were available only for squamous esophageal lesions. No glandular lesions of the esophagus were found, only 9% of cases had gastric cardia biopsies, and only 8% of the sponge samples had adequate glandular cellularity. Thus, although the glandular cytologic-histologic correlations of the balloon examinations have been given, this evaluation should be recognized as incomplete.

Nearly all the patients successfully swallowed the balloon, but 14% were not able to complete the sponge examination. This was surprising, because the encapsulated sponge was smaller in diameter and had a smoother surface than the deflated balloon, and previous studies of a similar sponge sampler in rural South Africa reported that only 1.3% and 1.7% of participants were unable to swallow the sponge.[31,32] The main reasons for incomplete sponge examinations appeared to be the patients' lack of familiarity with the procedure and the considerable flexibility of the sponge stylet. Most patients had seen or participated in a balloon examination before, but the sponge procedure was entirely new, so there was more patient anxiety and less patient understanding of what to do during the sponge procedure. In addition, the sponge stylet was not stiff enough for the technician to help push the capsule into the stomach, and patients accustomed to the balloon procedure expected this help. However, even with these difficulties more patients who attempted both screening methods preferred the sponge technique (43% vs. 31%). Greater patient familiarity with the sponge procedure and a stiffer sponge stylet probably would have reduced the number of incomplete sponge examinations and increased the patient preference for the sponge in this population. Different levels of patient acceptance of both samplers might be expected in different populations.

In most cases, the balloon produced a more cellular specimen than the sponge. More balloon samples had adequate squamous cellularity (by the criteria of the Bethesda system) and even when both specimens were considered technically adequate for evaluation the balloon specimen was nearly always the more cellular. The differences in specimen cellularity were even more striking in the sampling of glandular cells; 78% of the balloon samples, but only 8% of the sponge specimens, provided adequate numbers of glandular cells for evaluation. Two possible reasons for the superior cellularity of the balloon samples were that the balloon had a much more abrasive surface than the sponge and that it had a larger diameter and length and thus a larger surface area in contact with the mucosa. Another possible, but unconfirmed, reason for the sponge's poor sampling of glandular cells was that in some patients the stylet may have kinked and the capsule may never have reached the stomach. If this actually occurred, a stiffer stylet should help improve the sponge's gastric sampling.

The cytologic-histologic correlation analyses also suggest that the balloon sampled the esophageal mucosa better than the sponge. At all three diagnostic levels, the sensitivity of the balloon was greater than that of the sponge for detecting biopsy-proven squamous disease. The secondary analysis of the effect of sampler order also implied superior sampling by the balloon. In this analysis, the sensitivity of the balloon was similar when it was the first or second sampler used, but the sponge was three times as sensitive for DYS+ lesions when it was the initial sampler. Thus the balloon had no trouble collecting additional dysplastic cells after a previous sponge exam but the sponge was much less able to collect dysplastic cells after a previous balloon study.

In this study we examined four slides per balloon or sponge sample, primarily because this always had been the number of slides examined in previous balloon screenings. To evaluate the need for examining this many slides, we examined the detection rates of squamous esophageal lesions that we would have achieved if only one, two, or three slides per sample had been reviewed. For both the balloon and sponges samplers, most cases of squamous dysplasia or carcinoma were identified on the first slide reviewed, and this would have been true no matter which slide had been read first. However, there were clinically useful increments in cases detected on the second, third, and fourth slides and, for the balloon, the increments in sensitivity were statistically significant. Thus, our experience suggests that it is still prudent to examine (at least) four slides per case in screening exams.

Over the past 40 years there has been relatively little contact between Chinese and Western cytopathologists, and different systems of esophageal cytologic categorization have evolved.[18] In a recent comparison of Chinese and Western cytology readings, Western cytologic diagnoses appeared somewhat more accurate in identifying concurrent histologic lesions and predicting which individuals in a Linxian cohort would progress to future carcinoma,[18] so we decided to use Western cytologic categories and criteria in the current study. However, one potential limitation of this approach is that most Western esophageal cytologic criteria have been carried over from experience in analyzing samples from the uterine cervix, and there may be differences in the cytologic morphology that most accurately corresponds to similar histologic lesions in the esophagus and cervix. Thus, current Western criteria such as those in the Bethesda system may not be optimal for the esophagus. Indeed, the recent comparative study of Chinese and Western criteria suggested that the cytologic morphology associated with histologic mild squamous dysplasia in the cervix might be more indicative of moderate squamous dysplasia in the esophagus, and additional cytologic criteria might need to be developed to identify earlier esophageal lesions.[18] The current findings (Table 6) support this suggestion. For both samplers, inclusion of cases cytologically categorized as LSIL by the criteria of the Bethesda system prominently increased the sensitivity of detecting histologic HGD+, without greatly lowering the specificity of the cytologic diagnosis. Additional correlation studies of same-site esophageal brushings and biopsies will be needed to confirm and refine these observations.

Table 6 also shows some of the trade-offs inherent in trying to design an optimal screening program for a specific population. A previous follow-up study of asymptomatic patients in Linxian showed that individuals with biopsy-proven high grade (moderate or severe) squamous dysplasia were the only ones with a significantly increased risk for developing invasive squamous carcinoma within 3.5 years after endoscopy.[25] Thus a screening program in this population should try to maximize the identification of asymptomatic patients with these histologic lesions. Table 6 shows that increased detection of these patients could be achieved with the current cytologic samplers and diagnostic criteria by lowering the diagnostic cutoff that would send patients for endoscopy, but only at the cost of endoscopically examining additional patients, some of whom would not have high grade dysplasia or carcinoma. In our study, performing endoscopy on all patients with balloon diagnoses of HSIL-CA would have identified 16% of the patients with histologic HGD+ by examining 4% of those screened,

TABLE 7
Sensitivity and Specificity of Esophageal and Cervical Cytologic Examinations for Detection of Squamous Dysplasia and Carcinoma

	Esophageal balloon cytology[a]	Cervical Cytology[b]
Sensitivity	47%	63–79%
Specificity	81%	89–94%

[a] Current data.

[b] Data from Germain M, Heaton R, Erickson D, Henry M, Nash J, O'Connor D. A comparison of the three most common Papanicolaou smear techniques. *Obstet Gynecol* 1994;84:168–73.

performing endoscopy on patients with LSIL-CA would have detected 40% of those with HGD+ by examining 13% of those screened and performing endoscopy on patients with ASCUS, favor neoplasia-carcinoma would have identified 59% of those with HGD+ by examining 28% of those screened. In the extreme case, we could have achieved a 95% detection rate of HGD+ by endoscoping everyone with balloon diagnoses of Reactive-carcinoma, but only by examining 87% of those screened. Thus, designing the most appropriate screening protocol for a population requires deciding how best to balance the advantage of increased detection with the cost of performing endoscopy on larger numbers of patients, and this balance will be different for different populations.

Nearly all previous Chinese studies of the accuracy of the balloon sampler have been evaluations of the sensitivity of this technique for detecting esophageal carcinoma in symptomatic patients. Seven such studies have reported sensitivities of 88–99%,[9–12] although sometimes these sensitivities were achieved only after several balloon examinations in each patient.[11] The only previous evaluation of the balloon technique in asymptomatic Chinese patients reported sensitivities of 14–36% for a single balloon examination detecting concurrent, biopsy-proven carcinoma.[18] These figures are much more similar to the results of the current study. Studies of esophageal balloon cytology from other countries have reported sensitivities of 64–96% for detecting esophageal carcinoma in symptomatic patients already known to have carcinoma.[33–36] The only one of these studies to prospectively screen and then perform endoscopy on a cohort of asymptomatic subjects achieved a 100% sensitivity for identifying three carcinomas present in the 78 subjects examined.[36]

Japanese studies of the original Nabeya encapsulated sponge sampler have reported sensitivities of 76–95% for detecting esophageal carcinoma and sensitivities of 73–84% for detecting cardia carcinoma in hospital patients known to have these tumors.[14–16] However, a mass screening of 4000 subjects found no cases of esophageal carcinoma and only one case of cardia carcinoma. Although this one case of cardia carcinoma was confirmed by biopsy, the postscreening evaluation of the other subjects in this cohort was not reported, so the accuracy of the negative sponge examinations cannot be determined.[14–16] The only other evaluations of encapsulated sponge samplers have come from studies in rural South Africa. Using a retrievable foam disk compressed into a starch cachet, Burrell reported a sensitivity of 28% in 50 patients with advanced esophageal carcinoma.[37] He attributed the false-negatives in this series to the cachets being too large to pass through strictured lesions. Using a gelatin-encapsulated sponge similar to Nabeya's, Jaskiewicz et al. reported histologic confirmation of 9 carcinomas identified in a prospective screening of 752 asymptomatic subjects, but endoscopic evaluation was not available for 5 other cytologically diagnosed cases of carcinoma or for subjects with other cytologic diagnoses, so the sensitivity of the sponge technique in this population could not be determined. In a later similar prospective screening of 1336 asymptomatic subjects, Lazurus et al. reported a sensitivity of 90% and a specificity of 99% for detecting 10 cases of invasive squamous cell carcinoma, but endoscopic evaluation was again limited to cytology positive cases, and false negative cytologies were identified only by detecting cancer in a repeat cytology examination carried out 9–15 months later in 45% of the original patients.[32]

We believe that the true sensitivities of the balloon and sponge methods for detecting unknown esophageal squamous dysplasias and carcinomas in an asymptomatic screening population are far less than the 73–99% sensitivities reported for these techniques in symptomatic individuals. We think the results of the current study are probably the most accurate available estimates for the current capabilities of these cytologic methods in a screening setting. Except for possible differences in patient acceptance of the samplers, we believe our results should be applicable to all high risk populations, including those in Western countries.

To put the current findings in perspective, Table 7 compares our esophageal balloon results with the results of a recent similar cytologic-histologic correlation study of the uterine cervix.[38] In this study, a single cervical cytology examination was performed by one of three common sampling techniques immediately before colposcopy in 616 patients referred for a previous abnormal Papanicolaou smear, and the cytologic findings (test) were compared with the results of colposcopically directed biopsies (truth). It is clear from these data, as well as many other sources,[39] that a single Papanicolaou smear is considerably less than

100% sensitive for detecting biopsy-proven cervical dysplasia or carcinoma. However, it also is well documented that periodic cervical screening can dramatically reduce cervical carcinoma mortality.[40] This is possible because cervical carcinoma usually is a slowly growing disease, with a long preinvasive phase that can be detected by the Papanicolaou test and then treated successfully. The majority of cervical intraepithelial neoplastic lesions are detected in the first screening, and others are identified in later periodic examinations. Esophageal squamous cell carcinoma also is thought to be a slowly growing malignancy, with a long preinvasive phase,[40,41] so it also may be approachable by a strategy of periodic screening and treatment. Such a strategy should be able to reduce mortality even if the primary screening test is not 100% sensitive.

The fact that the sensitivity of the current esophageal balloon method is less than that of current cervical screening methods should not be surprising, considering that the balloon blindly samples a very large organ and the Papanicolaou smear samples a much smaller, visualized target area. However, even with the current balloon method, nearly 50% of the significant lesions in asymptomatic screenees should be detectable. Furthermore, the esophageal results reported in the current study clearly are baseline figures of currently available techniques, which can only improve with additional efforts to develop better samplers and more accurate esophageal cytologic criteria.

Both the balloon and sponge samplers have changed little in the past few decades, and additional research and development efforts should be able to significantly improve their diagnostic capabilities. The current study suggests several ideas for the design of an improved sampler: smaller, easier to swallow devices will be preferred; a relatively stiff delivery tube/stylet may be useful to assist swallowing and to ensure sampling of the gastric cardia; a surface material at least as abrasive as that of the current balloon may be required to produce adequate cellularity; having a larger contact area with the esophageal mucosa may increase diagnostic yield; and the ability to make smears directly from the sampler is desirable, especially in a field screening setting. Although most of the observations in this study were collected from the squamous esophagus, such improvements in sampler design also should be helpful for cytologic screening for adenocarcinomas of the esophagus and gastric cardia. Improvements in esophageal cytologic criteria also should be possible, with additional experience in cytologic-histologic correlation of esophageal samples.

Cytologic screening of asymptomatic high risk individuals remains a promising method for detecting curable squamous esophageal dysplasia and carcinoma. The sensitivity of current methods for identifying these lesions in asymptomatic adults is less than that previously reported for detecting esophageal carcinoma in symptomatic individuals, but is not far below the sensitivity of current cervical cytologic screening. In this study, the balloon sampler produced better squamous cellularity, much better glandular cellularity, and had a higher sensitivity for detecting squamous disease than the encapsulated sponge, but improvements in design should be possible for both samplers. Our data also suggest that the most accurate cytologic correlates of histologic lesions in the esophagus may not be identical to the cytologic correlates of similar histologic lesions in the cervix, so improvements in esophageal cytologic criteria also may be possible. Thus, although the current balloon and sponge techniques are not yet optimal for identifying curable squamous esophageal neoplasia, improvements in the samplers and cytologic criteria should be able to increase the sensitivities observed in this baseline study.

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